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THE FAST SYSTEM: Performance tests on the FAST TV diffractometer

by

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REPORT ON FAST PERFORMANCE TESTS

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INTRODUCTION

The FAST TV detector diffractometer is a device for measuring the x-ray diffraction patterns of protein single crystals. The benefits of such a detector, arising from better counting statistics and detector sensitivity compared with film, offer, amongst other things, an increase in both the accuracy of intensity measurements and the rate of data collection.

The FAST system is being made commercially by Enraf-Nonius, Delft and is based on a prototype developed at the MRC Laboratory Cambridge (Arndt and Gilmore (1979)).

During a visit to the factory of Enraf-Nonius, Delft (4-9 September) crystallographic data were collected by M. Papiz and N. van der Putten as part of the FAST system performance tests. Limited data were collected from crystals of ammonium bitartrate (used by Enraf to commission CAD-4 diffractometers) and rhodanese the protein crystal used by Enraf to commission the FAST.

At the time of the visit the FAST system was still under development and did not represent the final instrument which will be made available to Daresbury Laboratory. However the final system is only likely to differ in the improvements made in camera noise levels; the tests therefore place a lower bound on the accuracy of intensity measurements that can be made with the system.

Improvements had been made in one electronic board which was to reduce camera noise. However the improvements in noise levels coincided with an increase in the point spread function (i.e. PSF=8, c.f. 5) compared with the previous visit. It must be stated that although this observation may be significant the procedure for minimising the PSF by electronically tuning the detector had not been performed. The task of tuning the detector is performed by the electronic staff of Enraf-Nonius and would have required relinquishing the relevant electronic board for the duration of the visit. Because the tests were to discover the accuracy of the system in measuring intensities it was not thought important to insist on reducing the PSF for the period of the tests.

TEST 1

A spherical ammonium bitartrate crystal, diameter ≈0.3mm, space group $P2_12_12_1$, a=7.65Å, b=7.85Å, c=11.06Å was used to collect 18 pairs of equivalent reflections for agreement statistics. Data were collected with a sealed X-ray Cu Kα source operated at 26mA and 30kV. The camera and intensifier was set to the highest voltage gain of HV1i=HVCA=7. Two 20° rotation images were collected with an integration time of 73 seconds. Electronic noise was removed by subtracting from the image for 73 seconds with the x-ray shutter closed. The integration times were chosen to mimic the intensities of medium strength rhodanese reflections. Because of mmm symmetry in the diffraction pattern it was possible to find, by inspection, 18 pairs of symmetry related reflections (Table 1). These data produced an agreement R-factor on 1 of 5.1%. This R-factor is surprisingly good considering the effect of an unfavourably large rotation interval (20°) introducing large amounts of background noise.

TEST 2

The rhodanese crystal used was of cubic dimensions ≈0.3mm^3, space group C_2, a=156.0Å, b=49.0Å, c=42.2Å, β=98.6°.

A non-uniformity of response correction was generated from an image obtained by illuminating the detector with uniform x-radiation at an x-ray source to detector distance of approximately 1m. A spatial distortion correction table was created from measurements made from an image of an hexagonal lattice mask placed at the surface of the detector.

During data collection the detector to crystal distance was fixed at 51.2mm and the beam collimated to a dimension of 0.2mm. Data images were recorded in the rotation range of 0°-37.5° about the c axis in 2.5° intervals. Measurement times were 250 seconds per image. Noise subtracted images were corrected for non-uniformity of response, spatial distortion and interpolated to 100x100μ^2 pixel elements suitable for input into the film processing package MOSCO.

Data images were processed at Daresbury on the VAX/8750 with only minor modification to the MOSCO software. The final R.M.S. error in the crystal orientation matrix used to process the data was 0.03° in Bragg angle. Table 2 gives the merging statistics including the agreements between symmetry related reflections, R_{sym}(on 1).

For all data R_{sym} was 7.4%. This did not reflect the best that could be obtained from the detector since the dynamic range of the camera is less than that of the diffraction pattern. It would be therefore necessary to collect two images per rotation interval at short and long exposure times (equivalent to having more than one film per pack in the photographic data collection method). If the weakest reflections <50 are omitted leaving data within a dynamic range of 50:1 an overall R_{sym} of 5.3% is obtained. Perhaps a less arbitrary way of judging the data is to observe that the strongest reflections have an R_{sym} of 2%. This figure is better than the best that can be obtained with photographic data.

TEST 3

An equally important test is the comparison of data from the FAST system with that of the four circle diffractometer. Such a comparison could reveal undesirable systematic variations in the FAST measured intensities.

Rhodanese data from the CAD4 diffractometer (supplied by K Kalk, Groningen) were scaled to the FAST data. The scaling statistics are given below and show that within experimental errors the FAST data are in complete agreement with the diffractometer data.

	RF1 %	RF2 %	NR %	DR	No. of reflections
TOTAL	7.4	8.8	9.6	0.705	795
STRONG	4.9	4.2	5.4	-	108

$RF1 = \sum_H |I_{CAD}-I_{FST}| / \sum_H |I_{CAD}+I_{FST}|$ is the intensity agreement factor between the CAD4 (I_{CAD}) and FAST (I_{FST}) data summed over all indices (H=h,k,l).

$RF2 = \sum_H |I^+_{FST}-I^-_{FST}| / \sum_H |I^+_{FST}+I^-_{FST}|$ is the internal intensity agreement factor of the FAST data. I^+_{FST} and I^-_{FST} are Friedel related pairs of reflections.

Statistics have been calculated for the TOTAL and STRONG numbers of reflections which have measurements $I_{CAD}(H)$, $I_{FST}(H)$, $I^+_{FST}(H)$ and $I^-_{FST}(H)$.

$$NR = \frac{\sum(\sigma(I^+_{FST})+\sigma(I^-_{FST}))}{\sum(I^+_{FST}+I^-_{FST})}$$ and represents the counting statistics error inherent in the data measured on FAST. Hence, if (RF2-NR) was positive then this would represent residual noise not explained by Poisson statistics which could be attributed to either detector sensitivity fluctuations during the course of the measurements and/or systematic errors due to lack of a sample absorption correction. However, since NR>RF2 these contributions are obviously close to zero.

$D_R = \sum_H |F_A-F_F| / \sum_H |F_F^+-F_F^-|$ is the mean ratio of error between both data sets and within the FAST data set. $F_F = (F_F^+ + F_F^-)/2$, where F^+, F^- are Friedel related pairs of reflections.

It can be shown that for comparison purposes $DR < \frac{1}{\sqrt{2}}$ if the FAST data are to be in agreement with the diffractometer data.

DISCUSSION

It is hoped that the current improvements in detector noise levels will not be at the overall expense of the PSF. If we assume the best PSF of 5 so far obtained then this will mean 50x50 orders of reflections which could be measured simultaneously; this assumes a 0.2mm crystal (≈2 pixels), 2 pixels resolving neighbouring diffraction spots and neglecting beam divergence (i.e. 512/(2+2+5)≈57). This compares with a design specification of 100x100.

The implication of this is the reduction in the rate of data collected to one-fifth of the expected value. With phase 1 software this will have the consequence of reducing the size of proteins that could be studied and restricting the Bragg angle of data that could be measured (e.g. for 2Å resolution data a unit cell of 110Å, for 3Å data a cell of 165Å). However phase 3 software which utilizes fully the four circle geometry of the crystal goniostat combined with an increase in the crystal to detector distance will largely overcome these problems.

The detector noise level is low enough to produce R_{sym}'s somewhat better than the best film data as judged by strong reflection R_{sym}'s.

The dynamic range is smaller than expected ≈50:1 (cf. 100:1).

The best R_{sym}'s and dynamic range can only be obtained with the implementation of Phase 3 software which utilize the advantageous features of the FAST system. In the meantime it is encouraging that the present FAST system using film processing software compares very favourably with photographic data.

It should be remembered that the crystallographic measurements used to derive an R_{sym} will contain to a greater or lesser extent, systematic errors not associated with the detector such as sample absorption variations. We have attempted to minimize these particular effects by using spherical or cubic crystals.

References

Arndt, U.W. and Gilmore, D.J. J. Appl. Cryst. (1979), 12, 1-9.

TABLE 1: Reproduceability of intensity measurements for 18 pairs of reflections from a spherical crystal of ammonium bi-tartrate

Peak + Background area 20 x 20 pixels, Peak area 14 x 14 pixels

Pair No.	Intensity	sigma	Pair No.	Intensity	sigma
1	45454	296	10	18483	202
	46922	303		15584	192
2	43858	303	11	62256	322
	43324	310		72040	322
3	44225	288	12	42270	299
	50346	299		43472	300
4	44125	293	13	35184	308
	43554	295		40113	310
5	51356	292	14	76293	410
	53708	297		84899	413
6	22087	297	15	35970	367
	24824	294		37463	364
7	35886	328	16	92646	370
	39213	333		76313	336
8	14647	263	17	21354	227
	16864	264		23332	275
9	1350	301	18	49456	291
	5185	300		43431	278

$\langle I \rangle = 41597$ $\langle \sigma \rangle = 304$

$R_{sym} = 5.1\%$

$$R_{sym} = \frac{\sum_{H=1}^{H=18} \sum_{i=1}^{i=2} | \langle I(H) \rangle - I(H)_i |}{\sum_{H=1}^{H=18} \sum_{i=1}^{i=2} I(H)_i}$$

Table 2: Reproduceability of intensity measurements for a cubic rhodanese crystal

I_{max}	R_{sym}	N_{ref}	dmin(Å)	R_{sym}	N_{ref}
50	0.557	180	9.7	0.064	18
100	0.147	80	6.9	0.068	55
150	0.096	67	5.6	0.061	92
200	0.060	63	4.9	0.053	82
250	0.053	60	4.3	0.062	86
300	0.065	46	4.0	0.086	90
350	0.032	23	3.7	0.066	83
400	0.024	12	3.4	0.167	53
450	0.044	10	3.2	0.656	18
500	0.023	6			
550	0.058	2			
600	0.020	5			
650–2575	0.020	23			

maximum I = 2575

Unique no. of reflections = 3164

Unique no. of reflections with multiplicity > 1 = 577

% no. of reflections > 3σ = 71.5

overall R_{sym} = 7.4%

Overall R_{sym} rejecting I<50 = 5.3%